

AMENDMENTS TO THE SPECIFICATION:

1. Please replace the sixth full paragraph on page 4 (lines 25-29) with the following:

The present invention overcomes many of the aforementioned limitations. In this regard, the inventors of the present invention have surprisingly found that the gene that codes the **cytoplasmatic** **cytoplasmic** beta-actin protein and its derived products can be applied to taxonomic identification using samples of biological material deriving from a single species or a heterogeneous mixture of species and/or subspecies.

2. Please replace the paragraph bridging pages 4 and 5 (running from page 4, line 30, to page 5, line 5) with the following:

The present invention provides a method for identifying species and subspecies in a biological sample deriving from a single species or a heterogeneous mixture of species and/or subspecies, by means of the selective amplification of nucleic acid segments that code a target region of a macromolecule present in all the organisms concerned, for which, according to a first aspect, the object of the present invention is a method comprising a step whereby DNA is extracted from the sample; a step whereby

cytoplasmatic cytoplasmic beta-actin gene segments are amplified by PCR or an equivalent technique; and a step whereby the amplified segment is identified by comparing its size in base pairs with a pre-established standard of sizes and/or identifying the amplified segment by DNA sequencing and comparison of the resulting sequence with the specific sequence of each species or subspecies present on a computer database.

3. Please replace the second full paragraph on page 5 (lines 13-18) with the following:

Preferably, the regions to be amplified are divergent gene segments from the cytoplasmatic cytoplasmic beta-actin gene with DNA sequences with high evolutionary conservation between species and subspecies. And more particularly, the regions to be amplified are those which lie between the 3' sequence of the upstream exon and the 5' sequence of the downstream exon comprising the whole intronic sequence and part of the flanking exonic sequences.

4. Please replace the paragraph bridging pages 5 and 6 (running from page 5, line 31, to page 6, line 13) with the following:

The present invention provides the means of identifying a plurality of organisms in a single sample without having to use multiple probes that are specific to each of the species and subspecies that might be present in the sample. The method uses universal primers, which are valid for identifying any species or subspecies present in the sample without prior knowledge of the organisms that might be present. According to the invention, a composition of universal primers are used, which hybridise with the conserved regions of the **cytoplasmatic cytoplasmic** beta-actin gene, preferably with the sequences which lie between positions 1130-1191 and 1453-1473; 1453-1473 and 2041-2065; 2433-2459 and 2643-2680 and/or 26432680 and 2940-2960 (numbering in relation to the DNA sequence of the human locus HUMACCYBB Accession number M10277). The particular pairs of universal primers used are (1132-1151)
5'T000GCATGTGCAAGGCCGG3' (SEQ ID NO: 1) and (1474-1454)
5'CTCCATGTCGT000AGTTGG3' (SEQ ID NO: 2); (1453-1484)
5'ACCAACTGGGACGACATGGAGAAGATCTGGC3' (SEQ ID NO: 3) and (2063-2034)
5'TACATGGCNGGGTGTAAAGGTCTCAAAC3' (SEQ ID NO: 4), (2434-2463)
5'TGCCCTGAGGCCCTCTCCAGCCTCCTTC3' (SEQ ID NO: 5) and (2681-2643)
5'GGGTACATGGTGGTGCCGCCAGACAGCACNGTGTGGC3' (SEQ ID NO: 6); and (2643-2681) 5'GCCAACACNGTGTCTGGCGGCACCACCATGTACCC3' (SEQ ID NO: 7) and (2952-2932) 5'TCGTACTCCTGCTGATCCACATCTG3' (SEQ ID NO: 8).

5. Please replace the first and second full paragraphs on page 6 (two consecutive paragraphs, lines 14-36) with the following:

According to a second aspect, another object of the present invention is the use of DNA sequences of the **cytoplasmatic-cytoplasmic** beta-actin gene in biological samples deriving from a single species or from a heterogeneous mixture of species and/or subspecies, to identify the biological species to which the samples belong.

The **cytoplasmatic-cytoplasmic** beta-actin protein fulfils a number of criteria for achieving reliable identification. It is a ubiquitous protein in all the organisms concerned. **Cytoplasmatic-cytoplasmic** beta-actin is one of the six different isoforms of actin so far identified. Specifically, **cytoplasmatic-cytoplasmic** beta-actin is one of the two non-muscular cytoskeletal actins. Its function is to allow mobility and provide the cell with structure and integrity, being a majority component of the cellular contractile apparatus. For this reason, it is a fundamental protein for the cell's survival, which means that it presents exonic segments with a high evolutionary conservation between species. The degree of equivalence in its amino acid **sequence-sequence** between species is between 98% and 100%, sufficient to present highly conserved segments but also divergent segments in the non-coding parts of the gene to correctly distinguish between species that are closely related to one another. The nucleotide divergence corresponding, for example, to intron B of the species being studied (1216-1347 bp,

numbering in relation to DNA sequence of the human locus HUMACYBB Accession number M10277) is less than 25%. Segments that are highly conserved between the different species and subspecies make it possible to use primers that are common to all the species and subspecies, whilst divergent segments are the object of amplification using said primers, resulting in a different pattern of amplification for each species and subspecies.

6. Please replace the fourth full paragraph on page 7 (lines 19-27) with the following:

Figure 1 shows a diagram of the structure of the human **cytoplasmatic** **cytoplasmic** beta-actin gene. The boxes represent the exons (exon 1 to 6) and the continuous black line represents the introns (I, Intron A to E). Regions W, X, Y and Z correspond to regions which lie between the pairs of primers identified herein as SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and SEQ ID NO: 7 and SEQ ID NO: 8, respectively. These fragments (W, X, Y and Z) include DNA sequences that are divergent between different biological species and can be amplified using PCR using primers SEQ ID NO: 1 through SEQ ID NO: 8 as shown in figure 2.

7. Please replace the paragraph bridging pages 7 and 8 (running from page 7, line 33, to page 8, line 15) with the following:

Figure 3 Top of the figure: shows the partial amino acid sequence of the **cytoplasmatic cytoplasmic** beta-actin protein of three different species, Homo sapiens (man), Mus musculus (mouse) and Caenorhabditis elegans (nematode). The alignment between these sequences shows the high degree of conservation of the **cytoplasmatic cytoplasmic** beta-actin protein between species. The asterisks indicate 100% equivalence in that position between the species being compared. The numbering corresponds to the last amino acid shown according to the reference sequence in the GeneBank (refs: Hs: X00351. Mm: NM 007393.1. Ce: NM 073416.1). Middle of the figure: specifies the nucleotide sequence of the ends of exons 2 and 3 that flank intron B (W region) in said species. The exons show the nucleotide sequence in the three species being compared, divided into their corresponding codons and the amino acid residue that they code is shown below. The asterisks correspond to the nucleotide positions that are 100% conserved between the species being compared. Bottom of the figure: specifies the complete nucleotide sequence of intron B (divergent W region) in the three species being compared, to illustrate the divergence used for the identification of the species in this invention.

8. On page 9, after the second full paragraph and before the heading "DETAILED DESCRIPTION OF THE INVENTION" (after line 33 and before line 35), please insert the following new paragraph:

Figure 8 sets forth the entire DNA (nucleotide) sequence of the human cytoplasmic beta-actin gene, locus HUMACCYBB, GenBank Accession number M10277, verison M10277.1, GI:177967.

9. Please replace the only paragraph on page 11 (lines 1-12) with the following:

A second approximation then identifies the two bands obtained by sequencing their DNA. The bands were purified using Life Technologies' Concert Rapid PCR Purification System kit, so that their DNA could then be sequenced. The sequencing was performed cyclically in both directions with the same primers used in the initial PCR in accordance with the protocols and reagents of Applied Biosystems' ABI-Prism 310 automatic sequencing system. The two sequences that were obtained were used to interrogate a database of DNA sequences of the W region of the **cytoplasmic** **cytoplasmic** beta-actin gene of several species using the ClustalW program developed by the European Bioinformatic Institute of the EMBL (www.ebi.ac.uk) or an equivalent program that is available on the Internet (Figure 5). The comparisons resulted in a

100% equivalence of 1:5 and 2:8 in this case, confirming the source of the biological sample of origin, a mixture of goat and horse.

10. In addition to the foregoing amendments, please replace the current "Sequence Listing" consisting of three (3) pages, which appears at the end of the specification and before the claims, with the revised "Sequence Listing" consisting of five (5) pages submitted herewith.